

Article

Isolation and Antimalarial Activity of a New Flavonol from *Tithonia diversifolia* Leaf Extract

Talkmore Ngarivhume ^{1,*}, Anwar Noreljaleel ², Susanna L. Bonnet ² and Anke Wilhelm ²¹ Department of Chemical and Physical Sciences, Walter Sisulu University, Mthatha 5117, Eastern Cape, South Africa² Chemistry Department, University of the Free State, Bloemfontein 9300, Free State, South Africa; 2007096940@ufs4life.ac.za (A.N.); BonnetSL@ufs.ac.za (S.L.B.); WilhelmA@ufs.ac.za (A.W.)

* Correspondence: tngarivhume@wsu.ac.za

Abstract: An antiplasmodial activity-guided isolation was carried out on the dichloromethane extract of *Tithonia diversifolia* dried leaves. A total of five germacranolide type sesquiterpene lactones and a new flavonol, 3,6-dihydroxy-2-(4'-hydroxyphenyl)-7-methoxy-4H-chromen-4-one, were isolated. The flavonol reported an IC₅₀ above 6.00 μM against the chloroquine sensitive strain, NF54. The antimalarial activity of the *Tithonia diversifolia* dichloromethane leaf extract was attributed to orizabin and tagitinin C.

Keywords: *Tithonia diversifolia*; flavonols; antimalarial activity



Citation: Ngarivhume, T.; Noreljaleel, A.; Bonnet, S.L.; Wilhelm, A. Isolation and Antimalarial Activity of a New Flavonol from *Tithonia diversifolia* Leaf Extract. *Chemistry* **2021**, *3*, 854–860. <https://doi.org/10.3390/chemistry3030062>

Academic Editor: Anna Petruczynik

Received: 23 June 2021

Accepted: 20 July 2021

Published: 12 August 2021

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1. Introduction

An ethnobotanical survey indicated that *Tithonia diversifolia* is used as one of the traditional antimalarial remedies in Zimbabwe [1]. *Tithonia* is one of the genera of the *Asteraceae*, comprising about 11 species [2] and 13 taxa [3], and it originated from Mexico, Central America and Cuba [2]. In many African countries, such as the Democratic Republic of Congo [4], Kenya [5,6], Nigeria and Uganda [7], the use of the *rotundifolia* species in traditional medicines, livestock fodder, poultry feed, green manure, and field and storage pest management are widely acknowledged.

Extracts of this plant, particularly the leaf extracts, are reported to exhibit many biological activities, such as antimalarial, anticancer, antidiabetic, analgesic [8], anti-inflammatory, antidiarrheal, antihyperglycemic, cancer chemopreventive activities [9] and anthelmintic activity in goats in Rwanda [8]. Consequently, more than 150 compounds have been isolated from *T. diversifolia* [10]. Phytochemical studies revealed that *T. diversifolia* contains large amounts of sesquiterpene lactones, which constitute up to 3% dry weight of some *Asteraceae* species, e.g., *Helenium amarum* [11], flavonoids, diterpenoids [12,13], chromene, and flavones [14], among other minority compounds. These phytochemicals are most significantly concentrated in the leaves, followed by the roots and lowest in the stems [15,16].

Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure, most commonly known as the C₆-C₃-C₆ skeleton. They are synthesized by the phenyl propanoid pathway [17]. Based on their core structure, flavonoids can be grouped into different flavonoid classes, such as flavonols, flavones, flavanones, flavanols, anthocyanidins, isoflavones and chalcones. Flavonoids are often hydroxylated on positions 3, 5, 7, 3', 4', and/or 5', and some of the hydroxyl groups are methylated, acetylated, and even with sulphate conjugation [18]. Flavonoids are usually attached to sugar moieties through the O- or C-atom in plants [18].

Plants synthesize flavonoids as a response to microbial infection [17] or to environmental changes [19]. In plants systems, flavonoids help in combating oxidative stress and act as growth regulators [17]. Hydroxyl groups in flavonoids mediate their antioxidant effects

by scavenging free radicals and/or by chelating metal ions [17]. Fruits and vegetables are the main dietary sources of flavonoids for humans, along with tea and wine.

Many flavonoids are reported to have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some of them exhibit potential antiviral activities [17]. Kavitha et al. [20] report that flavonoids play an important role in maintaining the homeostasis of the central nervous system by modulating neuronal oxidative metabolism because they are strong inhibitors of enzymes that cause neuron degeneration. There is growing evidence that long-term ingestion of diets rich in plant polyphenols offer protection against the development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [21]. It is suggested that phenolic groups can act as electron sinks in living systems, forming relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components [21]. However, their bioavailability, metabolism, and biological activity depend upon the configuration, total number of hydroxyl groups, and substitution of functional groups about their nuclear structure.

2. Results and Discussions

2.1. Isolated Compounds from the DCM Crude Extract of *T. diversifolia* Leaves

A total of six compounds (Figure 1) were isolated from the antiplasmodial active fraction of the DCM crude leaf extract of *T. diversifolia*, comprising five germacranolide type sesquiterpene lactones and 18 mg of a new flavonol (Figure 2). The significant antiplasmodial activities of the DCM crude extract were attributed to orizabin and tagitinin C, which reported IC_{50} values of 2.28 μ M (0.83 μ g/mL) and 1.55 μ M (0.54 μ g/mL), respectively [1], while the flavonol reported an IC_{50} value above 6.00 μ M against the chloroquine sensitive strain, NF54. The cytotoxicity assessment of the flavonol was not done because it was not considered very active.

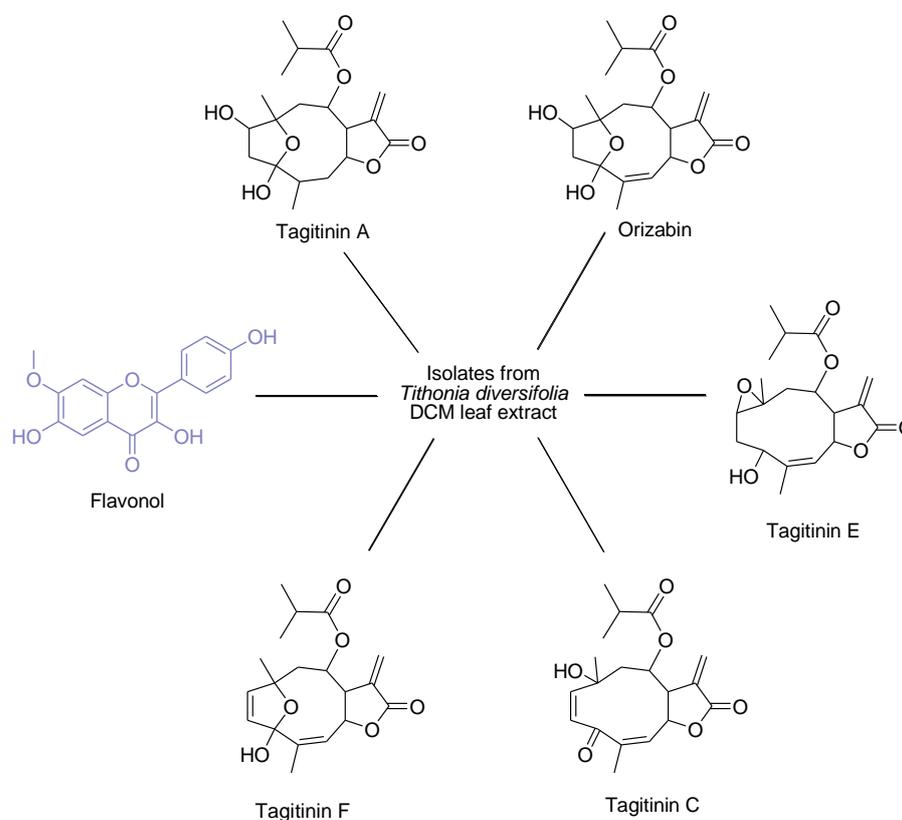


Figure 1. Compounds extracted from the antimalarial active TD DCM leaf fraction.

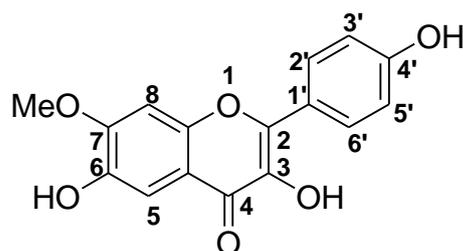


Figure 2. Structure of the flavonol.

The five sesquiterpene lactones in Figure 1 above have been reported on; therefore, no further studies on their physical properties were conducted. However, a search on the SciFinder database retrieved no match for the flavonol in Figure 2.

2.2. The Flavonol

The HRMS spectrum of the flavonol shows a molecular ion peak at m/z 301.0713 $[M + H]^+$ (calculated, 301.0712 $[M + H]^+$), corresponding to a molecular formula of $C_{16}H_{12}O_6$. The observed major fragment at m/z 247.0670, suggests a loss of 54 Da, and Figure 3 shows the proposed fragmentation pattern. The loss of CH_4 and CO [22] was not observed in the HR-ESIMS, despite the presence of peaks at m/z 284.0 and 256.2 in LRMS negative mode. The flavonol decomposes before melting.

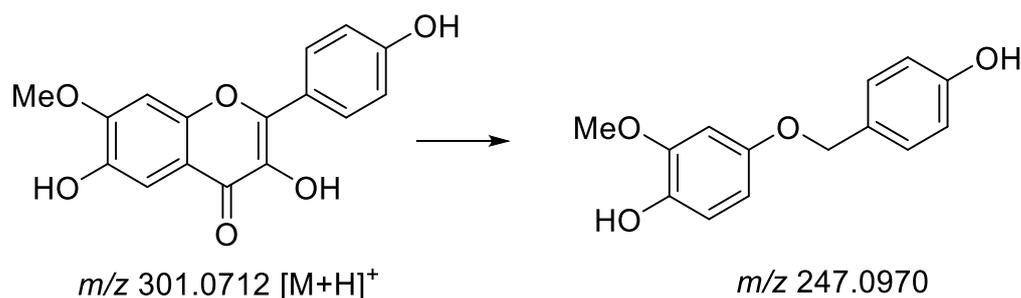


Figure 3. Fragmentation pattern for the flavonol.

The FTIR spectrum of the flavonol showed characteristic absorbencies of the carbonyl group at 1755 cm^{-1} [18], double bond stretching from 1552 to 1650 cm^{-1} , various C–O stretches from 1000 to 1295 cm^{-1} , hydroxyl groups at 3347 cm^{-1} and methyl stretches at 2922 cm^{-1} .

The ^1H NMR spectrum (Figure 4) shows an AA'BB' aromatic system with H-2'/H-6' at δ_{H} 7.92 (1H, d, $J = 8.8$ Hz), and H-3'/H-5' at δ_{H} 6.92 (1H, d, $J = 8.8$ Hz) (Table 1), respectively, while protons 5 and 8 resonate as two singlets at δ_{H} 6.77 and δ_{H} 6.59, respectively. The methoxy resonance is observed at δ_{H} 3.75 (3H, s) [18], and the OH proton is assigned at δ_{H} 13.07 (1H, s). The COSY spectrum shows a correlation between the H-2'/6' (δ_{H} 7.92) with H-3'/5' (δ_{H} 6.92).

The ^{13}C NMR spectrum shows a system with 14 carbons, while the APT experiment indicates 9 carbons in positive mode (8 quaternary carbons and one C=O) and 5 carbons in negative mode (1 methoxy group and 4 methine groups (CH)).

The 2D HSQC spectrum indicates that H-8 (δ_{H} 6.59) correlates with the carbon atom at δ_{C} 102.8, H-5 (δ_{H} 6.77) with the carbon atom at δ_{C} 94.7, H-3'/H-5' with the carbon at δ_{C} 116.4 and H-2'/H-6' with the carbon at δ_{C} 128.9.

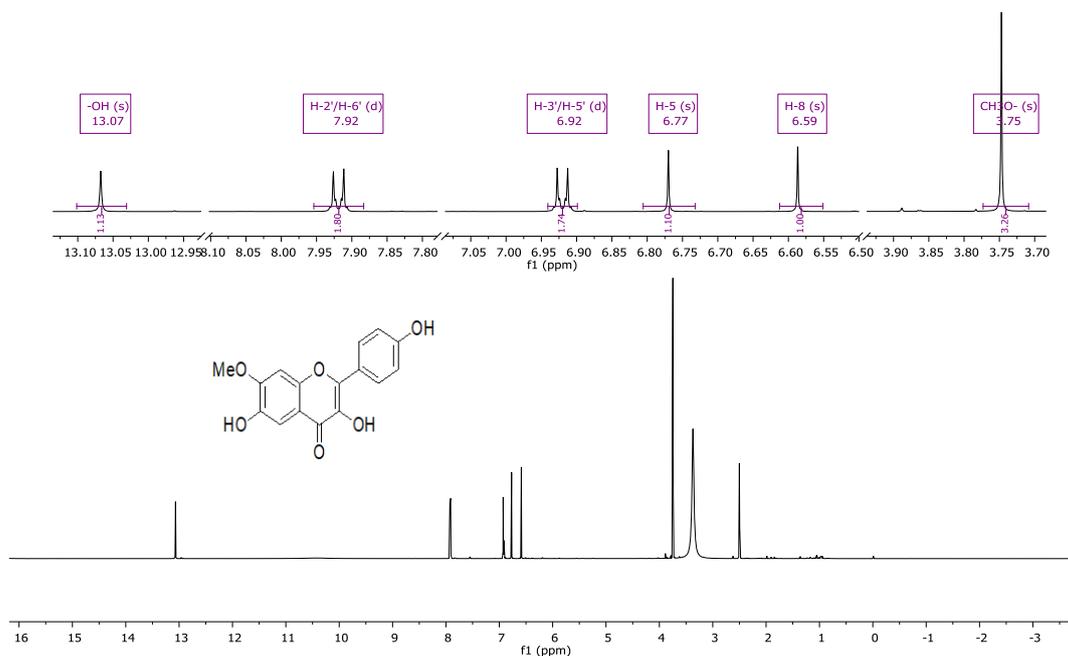


Figure 4. ^1H NMR spectra of the flavonol.

Table 1. ^1H and ^{13}C NMR data for the flavonol.

Position		^1H NMR/ δ (ppm) (600 MHz, $\text{DMSO-}d_6$)	^{13}C NMR/ δ (ppm) (150 MHz, $\text{DMSO-}d_6$)
1	-	-	-
2	-	-	164.2
3	-	-	153.2
4	-	-	182.5
4a	-	-	157.8
5	H-5	6.77 (1H, s)	94.7
6	-	-	104.5
7	-	-	131.8
8	H-8	6.59 (1H, s)	102.8
8a	-	-	152.8
1'	-	-	161.6
2'	H-2'	7.92 (1H, d, $J = 8.8$ Hz)	128.9
3'	H-3'	6.92 (1H, d, $J = 8.8$ Hz)	116.4
4'	-	-	121.6
5'	H-5'	6.92 (1H, d, $J = 8.8$ Hz)	116.4
6'	H-6'	7.92 (1H, d, $J = 8.8$ Hz)	128.9
-OMe		3.75 (3H, s)	60.4
-OH		13.07 (1H, s)	-

Figure 5 shows information deduced from the 2D HMBC spectrum, indicating that H-5 (δ_{H} 6.77) strongly correlates with the carbonyl carbon assigned as C-4, at δ_{C} 182.5, while H-8 (δ_{H} 6.59) slightly correlates with the same carbonyl carbon atom. The methoxy protons and H-8 correlate with the carbon at δ_{C} 131.8, assigned as C-7. Protons 3'/5' (δ_{H} 6.92) strongly correlate with C-4' at δ_{C} 121.6 and weakly correlate with C-1' at δ_{C} 161.6, while H-2'/H-6' (δ_{H} 7.92) strongly correlate with C-1' at δ_{C} 161.6 and C-2 at δ_{C} 164.2, and weakly correlate with the carbons of H-3'/H-5' at δ_{C} 116.4. The carbon at δ_{C} 153.2 does not correlate with any proton, indicating that it is C-3. The arrows indicate correlating atoms according to the HMBC spectrum. More Supplementary Material that was used to characterize the compound is available on MDPI website

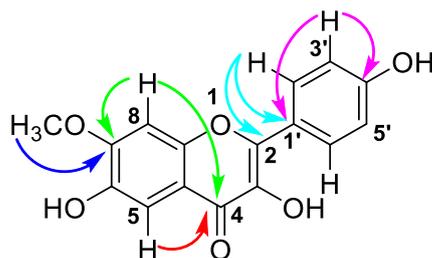


Figure 5. HMBC correlations of the flavonol.

A web-based search retrieved no matching responses for the flavonol. Its scientific name could be deduced as 7-methoxy-3,4',6-trihydroxyflavone or 3,6-dihydroxy-2-(4-hydroxyphenyl)-7-methoxy-4*H*-chromen-4-one.

3. Materials and Methods

3.1. Characterisation of Isolates

A 600 MHz Bruker Avance spectrometer (Fallanden, Switzerland) was used to record the ^1H NMR, COSY, HMBC, HMQC (600 MHz) and ^{13}C , APT (150 MHz) experiments in DMSO- d_6 ($\delta\text{H} = 2.50$; $\delta\text{C} = 39.51$) with TMS as the internal standard. Chemical shifts were expressed as parts per million (ppm) on the delta (δ) scale, and coupling constants (J) are accurate to 0.01 Hz. High-resolution mass spectral data (HRMS) were collected using a Waters Micromass LCT Premier TOF-MS (Milford, USA), while low-resolution mass spectra (LRMS) were recorded on a Sciex 4000QTRAP hybrid triple quadrupole ion trap mass spectrometer (Foster City, CA, USA), and the infra-red (FTIR) spectra were recorded on a Perkin Elmer Spectrum One FT-IR (Shelton, CT, USA).

3.2. Crude Extracts

Fleshy leaves of *Tithonia diversifolia* were air-dried in the shade, and then finely grounded. A sample of the powdered leaves was sequentially soaked in hexane, DCM and then in a mixture of DCM and ethyl acetate (9:1 *v/v*) on a shaker. The sample was repeatedly soaked in each solvent over several nights until there was a significant loss of color and the major eluents were diminishing on the TLC. The three fractions were concentrated on a Rotavapor at 40 °C and then transferred into separate, labeled vials, which were left to dry in a fume hood.

3.3. Antiplasmodial Activity Assessment

The chloroquine-sensitive NF54 strain of the malaria parasite *Plasmodium falciparum* was cultured in vitro [23]. The antiplasmodial activity of the various extracts was determined using the tritiated hypoxanthine incorporation assay [24]), where chloroquine and quinine were used as the reference antiplasmodial agents. At least three independent experiments were performed, from which the mean and standard deviation were determined.

3.4. Isolation of Antiplasmodial Active Fractions

The DCM fraction was further fractionated on a silica gel column with hexane-ethyl acetate (H-EA) solutions (15:1; 10:1; 5:1; 3:2 and 2:3 (*v/v*)). The column was followed by TLC. Sub-fractions 9 (0.59 g) and 10 (1.67 g) of the DCM from eluents H-EA 5:1 and 3:2 had very similar TLC profiles, and reported the highest antimalarial activities as reflected by IC_{50} values of 0.31 ± 0.07 and 0.62 ± 0.04 $\mu\text{g}/\text{mL}$, respectively, and an average inhibition of 53.7 %, which were sustained even at a low concentration of 0.5 $\mu\text{g}/\text{mL}$ [1]. The two sub-fractions were combined (1.60 g) and then further subjected to silica gel column chromatography with DCM-EA eluents (20:1; 12:1; 7:1; 5:2; 3:2, and 2:3 (*v/v*)). All the isolates, except the flavonol, were cleaned by re-crystallization in ethyl acetate and hexane mixtures [1]

The major sub-fraction from eluent DCM-EA 7:1 was re-dissolved in ethyl acetate after rotary evaporation, and on standing, light yellowish-green amorphous particles separated, which were filtered off. On further standing, two more crops were harvested. The precipitate did not dissolve in ethyl acetate, chloroform, methanol or ethanol, but dissolved in DMSO. The isolate was dissolved in DMSO- d_6 , and an ^1H NMR was run, which indicated that it was a pure compound [1].

4. Conclusions

A combination of gravity chromatography and re-crystallization afforded five germacranolide type of sesquiterpene lactones and a new flavonol from the DCM fraction of the *T. diversifolia* leaf extract. Antiplasmodial activity of DCM crude extract of *T. diversifolia* leaves is mainly due to orizabin and tagitinin C.

The six isolated compounds may further be assessed for GABA_A and Acetylcholinesterase inhibition effects. Additionally, the flavonol could be tested for anticancer and antidiabetic activities because flavonoids exhibit antioxidant and anticancer activities. Furthermore, flavonol could be severally functionalized and the products assessed on various diseases.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/chemistry3030062/s1>, S1: NMR spectra; S2: FTIR spectrum of powder; S3: Mass spectra.

Author Contributions: Conceptualization, T.N.; methodology, T.N.; software, S.L.B.; validation, S.L.B., A.W. and A.N.; formal analysis, T.N., A.N., S.L.B. and A.W.; investigation, T.N.; resources, S.L.B. and A.W.; data curation, S.L.B.; writing—original draft preparation, T.N.; writing—review and editing, S.L.B. and A.W.; visualization, A.N.; supervision, S.L.B. and A.W.; project administration, S.L.B.; funding acquisition, S.L.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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